Technical Report No. 65

TEMPORAL PATTERN OF GENE ARRANGEMENT FREQUENCY IN ALTITUDINAL POPULATIONS OF *DROSOPHILA IMMIGRANS* ON MAUNA LOA, HAWAII

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U. S. International Biological Program

May 1975

ABSTRACT

The chromosomal polymorphism of D. immigrans from three different elevations on Mauna Loa, Hawaii was studied from 1971 to 1973. Three inverted gene arrangements in the second chromosome were common at most times of these years. The frequencies of arrangement A in the left arm of this chromosome showed a "spring-summer-winter" pattern of seasonal change in 1973 when collections were made almost monthly, but the other two arrangements in the right arm showed no perceptible changes. The A arrangement also showed significant altitudinal variation in frequency in 1971. The frequency pattern of gene arrangement A was correlated with dry condition of the environment.

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INTRODUCTION

Natural populations of the cosmopolitan species Drosophila immigrans are known to have three universally distributed inversions, all of which are located on Chromosome 2. Though the number of chromosomal polymorphisms in this species is relatively scant, observations by different workers on continental populations have shown that considerable differences exist not only among the inversion types, but also in heterozygous tendencies among geographic populations (Freire-Maia, Zanardini and Freire-Maia 1953; Brncic 1955; Gruber 1958; Hirumi 1961; Toyofuku 1961; Paik 1973). Geographic variations in chromosomal inversion frequencies reflect the operation of natural selection if populations are not subject to harsh fluctuation in size or genetic drift. Recently, Richmond and Dobzhansky (1968) and Paik and Sung (1974) have found different patterns of inversion frequencies among islands of the Hawaiian archipelago, suggesting that coadaptive processes in inversion systems may be involved as well. Further, their island populations display much greater amounts of inversion polymorphism than do the continental populations, a phenomenon opposed to situations generally found by others in different species for comparisons between continents and islands (Dobzhansky 1957; Ayala, Powell and Dobzhansky 1971). The amount of inversion polymorphism in a population is related at least theoretically to the breeding structure of the population and to the nature of its environment.

The present report is intended to provide an account of the pattern of temporal variation in gene arrangement frequencies in natural populations of Drosophila immigrans on Mauna Loa, Hawaii and the relationship of these patterns to climatic variations.

MATERIALS AND METHODS

Collections of flies have been made by exposing plastic buckets, 33 cm diam. x 38 cm deep, each containing about one kg of yeasted banana as bait. These traps were placed on shaded ground and the flies attracted were taken mostly with the aid of a collecting net. Five to seven traps were exposed at each site for four days; early morning and late afternoon collections were made daily for three days beginning on the second day.

The samples of D. immigrans were taken at sites located at 1220 m (Kipuka Puaulu), 1550 m and 2040 m chosen near the Mauna Loa Strip Road, Volcanoes National Park. This general area affords a wide range of ecological conditions, from dense

savanna or "kipuka" forests at the low elevation and mountain parkland tree vegetation at the intermediate, to open subalpine scrub forests at the high elevation. General descriptions of biotic characteristics of this area can be found in Doty and Mueller-Dombois (1966). First samples at these sites were taken in April and the second samples in December 1971; third samples were taken in October 1972. In 1973 collections were made in April, July, August, September, October and December. Several climate variables were measured during the Drosophila collection period at or near our study sites by the US/International Biological Program, Island Ecosystems IRP, and these data are found in Bridges and Carey (1973, 1974).

The captured flies were brought into the IBP lab on Mauna Loa for identification, and females of D. immigrans were separately placed in the food vials (Spieth 1966) for air transportation to our lab in Honolulu. These females were then transferred to individual culture vials containing standard corn meal-molasses-agar medium mixed with a small amount of wheat germ. When the larvae had become fully grown, the salivary gland chromosomes of one larva per wild female were examined in aceto-lacto orcein squashes. This method identifies the chromosomal phenotype of a random sample of fertilized eggs which normally would have been laid in nature by individual females. Frequencies of chromosomal phenotypes were determined by the identification of both homokaryotypes and heterokaryotypes, and these will be referred to as the "frequency of gene arrangement."

RESULTS

Four inversions have been found in the Mauna Loa populations. Of these, three are identical to those found by other workers in continental populations and the fourth variant is probably an unreported, new inversion of Chromosome 2. Following the conventional notations for the cosmopolitan inversions, the median inversion of the left arm of Chromosome 2 will be designated as A, the subterminal one of the right arm as B, and the proximal one of the right arm as C in the tables, figures and text of this paper. The new variant is a complex inversion which overlaps the break points of the C inversion; this will be designated hereafter as D.

The frequency data of the three common gene arrangements are reported in Table 1, and represented graphically in Figures 1.1, 1.2, and 1.3. The percentage frequencies of separate gene arrangements given in the table have been compiled from the numbers

TABLE 1. Frequencies of gene arrangements A, B and C for the years 1971, 1972 and 1973 at 1220, 1550 and 2040 meters on Mauna Loa

Collection	1220 m				1550 m				2040 m			
	No. of Chrom's	%Gene arrangement A	B	C	No. of Chrom's	%Gene arrangement A	B	C	No. of Chrom's	%Gene arrangement A	B	C
April 1971	134	14.2	11.2	11.2	202	24.7	9.4	6.4	52	21.1	1.9	7.7
Dec.	454	15.9	9.7	9.0	180*	23.3	8.9	3.9	-	-	-	-
Oct. 1972	500*	19.6	6.8	9.0	124	13.7	1.6	7.3	76	22.4	5.3	6.6
Mar. 1973	406	14.3	6.4	7.1	-	-	-	-	-	-	-	-
April	268*	17.5	7.1	6.7	262	14.9	3.8	7.2	-	-	-	-
July	234	19.7	7.3	10.7	26*	23.1	7.7	3.8	-	-	-	-
Aug.	302	20.8	4.3	7.0	94*	20.2	7.4	5.3	16	18.8	18.8	6.3
Sept.	216*	19.4	8.8	7.4	14	28.6	7.1	-	10	30.0	10.0	20.0
Oct.	292	16.4	6.2	6.2	70*	12.9	5.7	8.6	14	21.4	7.1	-
Dec.	292*	11.6	5.1	9.6	218	15.1	5.0	6.9	44	11.4	2.3	6.8
Total	3098	17.0	7.1	8.2	1190	18.4	6.1	6.3	212	19.8	5.2	7.1

* Indicates the presence of arrangement D in the corresponding samples.

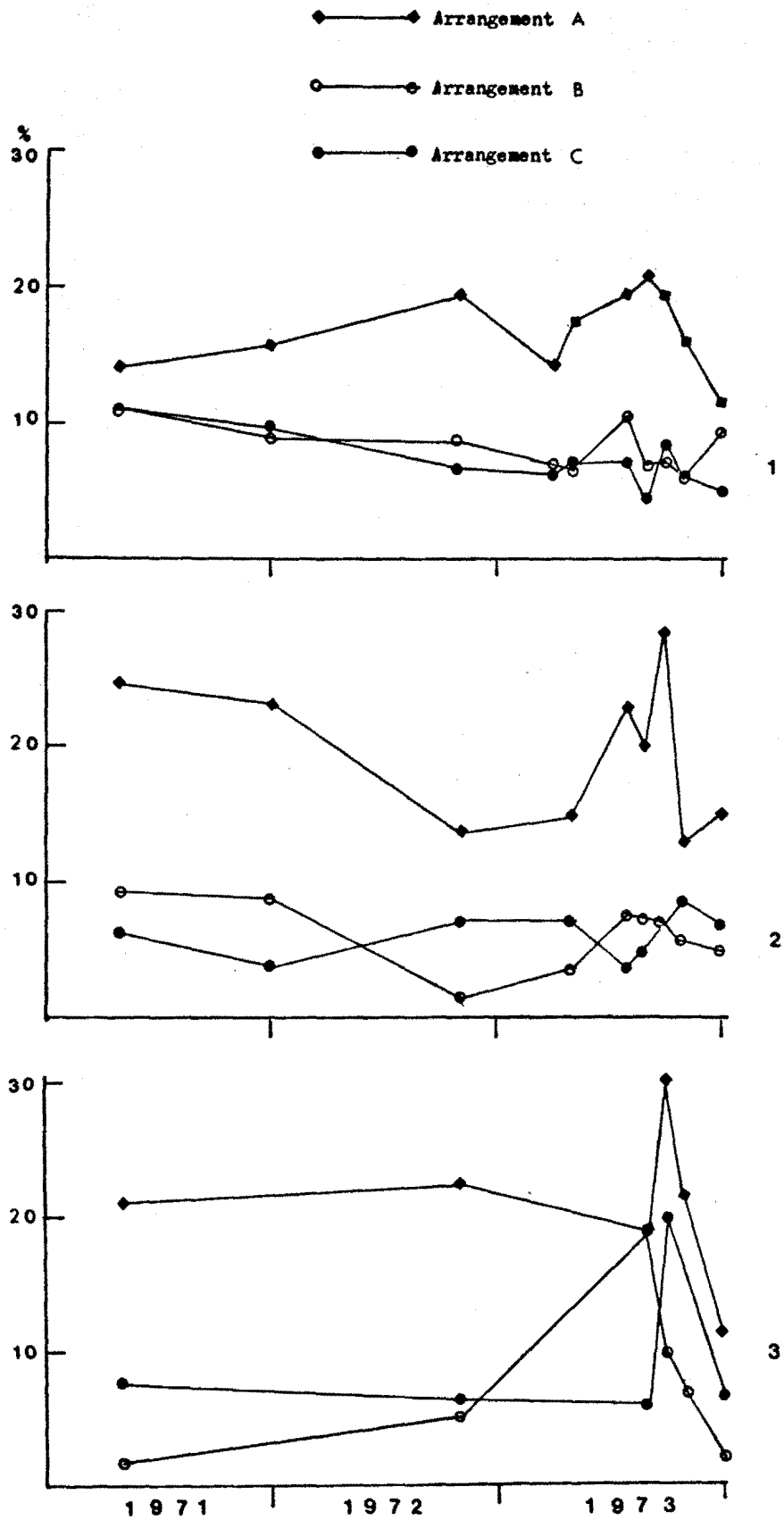

FIG. 1.1, 1.2 and 1.3. The frequencies of arrangements A, B and C plotted against the date of collection at 1220 m (1), 1550 m (2) and 2040 m (3).

of single, double and/or triple heterokaryotypes and of homokaryotypes (always single) which have been identified in the larval samples. The D arrangements have been found sporadically during the three years at the two lower elevations at a total frequency of 0.2%; they are too rare to be included in the table.

A general comparison of frequencies in Table 1 shows that in all collections arrangement A was at the highest frequency, followed by either the B or C arrangement. This is more apparent on examination of Figure 1.

The two samples taken eight months apart in 1971 at 1220 m were very similar in the relative frequencies of the three gene arrangements as shown in the table; the same constancy was shown at 1550 m. Only one collection was made at 2040 m so no comparison can be made for this time interval. Considering the April and December samples together, the A arrangement frequency at 1220 m is significantly lower than those from the higher elevations ($P = 0.004$ by chi-square); a comparable P value for the C arrangement shows the borderline of significance (P is just below 0.05); for the B arrangement it is equal to 0.5-0.1. The stratification by elevation for arrangement A is at least meaningful.

In comparing the 1971 and 1972 frequency data, no appreciable variations from year to year were seen except for the very low frequencies of arrangements A and B found at 1550 m in 1972. The frequencies of A and B at the intermediate elevation dropped 10 and 7%, respectively, in October 1972 from their combined frequencies of April and December in 1971; these differences are statistically significant ($P = 0.007$ for the A arrangement and $P = 0.003$ for the B arrangement by hypergeometric test). The 1.6 percent B frequency in the October 1972 sample at 1550 m is unusual since it is the lowest found for this arrangement in this area. The variations between elevations were tested for significance, and it was found that the difference in the B arrangement frequencies between 1220 and 1550 meters is the only one which is statistically significant ($P = 0.01$ by hypergeometric test).

The 1973 monthly frequency data in Table 1 indicate that pronounced changes occurred in the frequencies of arrangement A at all elevations. From Figure 1.1, it can readily be seen that the frequency at 1220 m was relatively low in the "spring" period from March to April, and increased considerably in the "summer" period from July to September, and returned to approximately its spring value during the "winter" period from October to December. The overall changes from March to December are significant ($P = 0.03$ by chi-square), and the result suggests that the changes follow a seasonal trend. Looking at Figure 1.2, the spring-summer-winter pattern was also

discernible at 1550 m; the hypergeometric tests indicate that the P value is slightly below 0.05 for the comparison of the April frequency with the combined frequency of the summer samples. Comparing the summer frequency with that of the winter samples, P is 0.04. Visual inspection of Figure 1.3 is consistent with the above observations in that the pooled frequency of arrangement A (23%) at 2040 m from the August and September samples is appreciably higher than the October-December frequency (14%), although the difference is not significant (P = 0.15 by hypergeometric test). This statistical insignificance is probably the result of the inadequate sample sizes at the same time when large changes in frequency occurred.

In contrast to the A arrangement, the 1973 monthly variations recorded for the B and C arrangements were generally small and showed no definite trends of change. None of the changes are significant for any of the two arrangements or for any of the three elevations, indicating that the frequencies of these arrangements remained relatively stable at all elevations throughout the year.

In comparing the pooled frequencies of the three arrangements of the April-September 1973 samples from 1220 m with those from 1550 m, the hypergeometric tests have revealed that the value of P for each arrangement is always above 0.13. Similarly, for the between-elevation comparison of the pooled frequencies of the arrangements from the October-December samples, P for each arrangement is always greater than 0.40. The monthly samples taken from the high elevation of 2040 m are too small to warrant their inclusion in the present comparison. The present suggestion is that the stratification of gene arrangement frequency by elevation was not present in 1973 for any of the three gene arrangements.

DISCUSSION

Temporal Changes in Gene Arrangement Frequencies

Frequencies of D. immigrans gene arrangements studied almost monthly in 1973 on Mauna Loa show "spring-summer-winter" pattern of seasonal changes in gene arrangement A. The pattern was quite apparent at 1220 m where the population appears to maintain a consistently high population size (large numbers of flies were caught in every collection at this site). A similar trend was also seen at the sites above 1550 m, but the pattern was not as evident at these elevations as at the low elevation. However, this result is not unexpected, since the higher elevations do not

always maintain large populations, i.e., the populations generally pass through "bottle-necks" of low abundance. For this reason, the relatively poor representation of the regular pattern of changes observed at the higher elevations must be due to sampling errors and/or drift effects. The above results suggest that the seasonal frequency changes in the A arrangement are probably adaptive patterns. Furthermore, the relatively rapid changes observed in the A arrangement may imply that selection operates directly on the carriers of this gene arrangement rather than upon some second order consequence(s). The seasonally "flexible" polymorphism of the A arrangement in the Mauna Loa populations of D. immigrans appears to be essentially similar to the situations found in other well-studied polymorphic Drosophila species (in D. funebris, Dubinin and Tiniakov 1945; Borisov 1969; in D. pseudoobscura, Dobzhansky 1943, 1956; Dobzhansky and Ayala 1973; in D. persimilis, Dobzhansky 1956).

Unlike the A arrangement, the frequencies of arrangements B and C do not show any regular, significant changes but have remained essentially constant during the period of 1973 at all elevations studied. The general constancy of these arrangements suggests that they are capable of responding to the seasonal variations without changing their frequency in the populations. The relative constancy of these arrangements during the entire periods of 1971 to 1973 further supports the present argument. These findings present a "rigid" polymorphic pattern similar to that found in D. robusta (Carson and Stalker 1949; Carson 1958), although this species showed seasonal changes in the frequencies of some X-chromosome gene arrangements of a local population (Levitan 1957). Tonzetich and Ward (1973) indicated that in populations of D. melanica seasonal responses in frequency occurred in autosomal gene arrangements but not in X-chromosome arrangements. According to Dobzhansky (1962), the flexible and rigid pattern of inversion polymorphisms are different adaptive means which are not mutually exclusive and would be utilized by a species or population through selection whenever opportunities arise.

The elevational stratification in the A arrangement frequency in 1971, based on two collections at 1220 and 1550 m sites, is also interesting. The frequencies of A observed at the two higher elevation sites (21-25%) are distinctively greater than they were in the same months observed at the low elevation (14-16%). While a precise reason for this difference cannot be ascertained at present, the differences occurred when the populations were obviously at high numerical levels, and the reality of the difference is statistically assured. Moreover, the monthly frequency data of 1973 have already shown, as we argued previously, that the A arrangement is likely to

react to seasonal changes in the environment. Considering also the relative constancy in the frequencies of the two right arm gene arrangements in 1971 at all elevations, drift effect on the variations of the A arrangement can be expected to have had little importance. All this information suggests that the between-elevation differences noted for the 1971 frequency of A arrangements may be a reflection of variations in general environment through the transect.

The sharp decrease in the frequencies of the A and B arrangements in 1972 at the 1550 m site poses another interesting problem. Changes in the frequency of the A arrangement were discussed earlier. The changes in the B arrangement frequency, however, were not observed to be significant during other periods. In particular, the 1972 frequency of B arrangements at 1550 m (1.6%) was so low that this gene arrangement got into elevational stratification in a statistical sense. Unfortunately it is uncertain whether or not the changes are the result of selection due to alterations in the environment, since no samples were taken on corresponding months between 1971 and 1972. However, the results obtained here at least warrant some further examination. It is possible, for example, that the 1972 changes found at 1550 m from the October sample might also be explained by considering that this sample was the immediate offspring of the small number of flies which had survived through the "bottle-neck" of low population size in the summer, because our population size estimates give some inferences that perturbation of the environment is relatively severe, particularly in summer, at the higher elevations.

Relation to Climate Variables

The patterns of change in the frequency of arrangement A may be attributable to the effects of climate on the physical and/or biotic environment, although it is difficult to identify the particular environmental factors responsible. Dobzhansky (1952) found that particular gene arrangement in natural populations of D. pseudoobscura and D. persimilis showed significant increases in frequency during a period of drought, while the reverse was true for other gene arrangements. Strickberger and Wills (1966) have suggested that the adaptive values of different gene arrangements are determined by different climatic factors, based on their study on the relationship between the frequencies of specific gene arrangements in D. pseudoobscura and temperature and rainfall. Tonzetich and Ward (1973) found that carriers of a particular gene arrangement in D. melanica showed greater survival than did

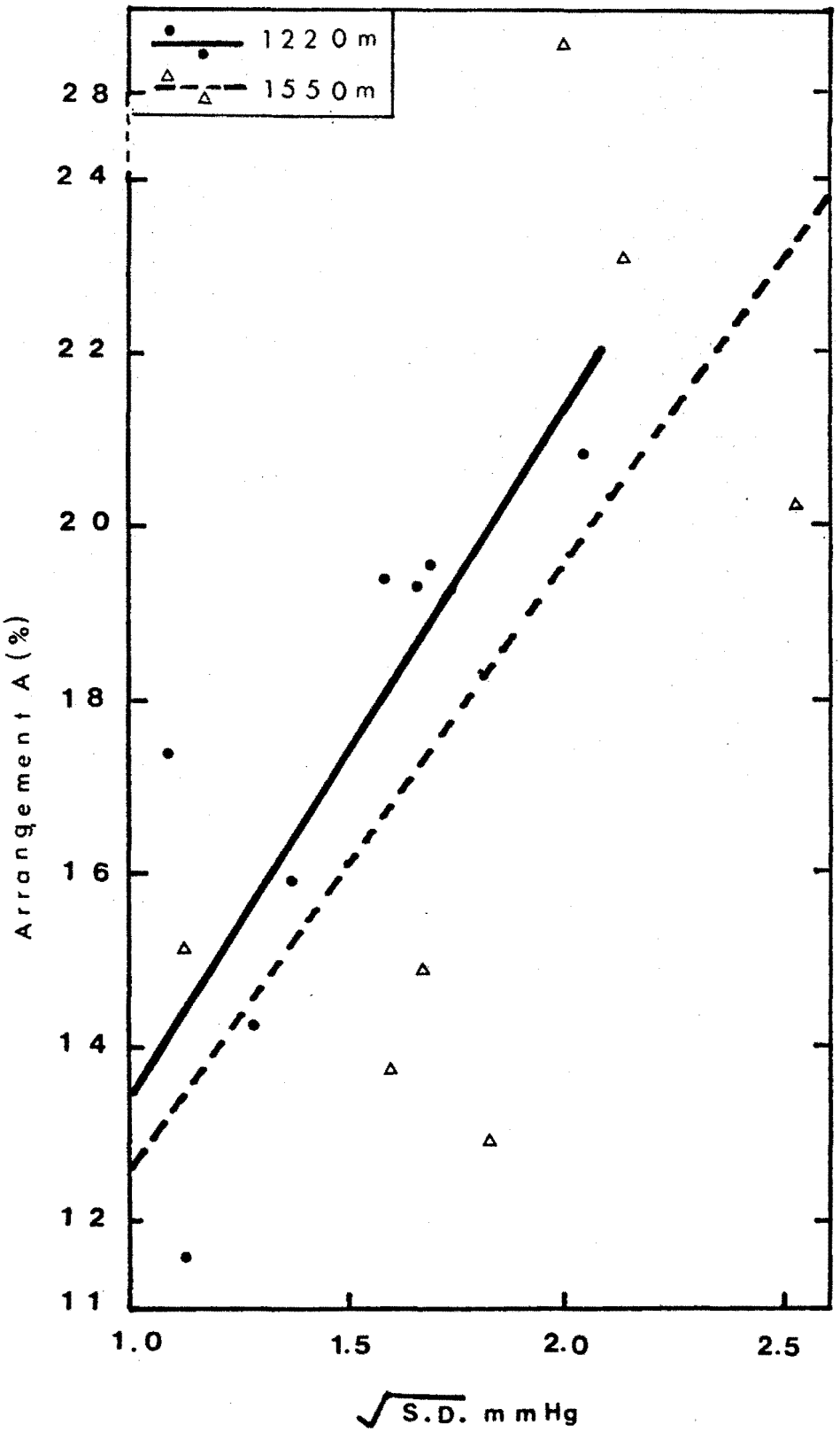

FIG. 2. The correlation between the frequency of gene arrangement A and the square-root of the saturation deficit ($\sqrt{S.D.}$) at sites 1220 m and 1550 m.

TABLE 2 The distribution of the daily minimum temperatures (C°) of 1971 to 1973 near 1550 m. The figures given are percentage of days that temperatures occurred in the various ranges during the month

Year / Temp. / Month	1971					1972					1973				
	≤0	1-4	5-8	9-12	≥13	≤0	1-4	5-8	9-12	≥13	≤0	1-4	5-8	9-12	≥13
Jan.	19.4	35.5	38.7	6.5	-	-	46.7	43.3	10.0	-	-	25.8	54.8	19.4	-
Feb.	30.8	30.8	34.6	3.8	-	-	31.0	48.3	20.7	-	-	48.1	33.3	18.5	-
Mar.	19.4	29.0	41.9	9.7	-	-	9.7	58.1	32.3	-	-	-	9.7	90.3	-
Apr.	-	13.3	60.0	26.7	-	-	-	26.7	63.3	10.0	*	*	*	*	*
May	-	41.9	58.1	-	-	-	-	25.0	75.0	-	-	41.2	41.2	17.6	-
Jun.	3.3	33.3	56.7	6.7	-	-	-	10.0	86.7	3.3	-	10.0	50.0	40.0	-
Jul.	-	41.9	41.9	16.1	-	-	-	19.4	51.6	29.0	-	4.0	28.0	60.0	8.0
Aug.	-	-	58.1	41.9	-	-	-	48.4	48.4	3.2	-	3.2	61.3	29.0	6.5
Sep.	-	-	13.3	76.7	10.0	-	-	30.0	70.0	-	3.3	23.3	36.7	36.7	-
Oct.	-	-	41.9	48.4	9.7	-	-	45.2	54.8	-	-	3.2	32.3	58.1	6.5
Nov.	-	6.7	50.0	40.0	3.3	-	13.3	46.7	40.0	-	-	-	36.7	63.3	-
Dec.	-	35.5	61.3	3.2	-	-	12.9	71.0	16.1	-	-	6.5	58.1	29.0	6.5
Pooled	5.9	22.3	46.5	23.4	1.9	-	9.5	39.5	47.1	3.9	0.3	13.7	40.4	43.1	2.6

* Shows lack of the weather data.

months at 1550 m and 1220 m was analyzed for heterogeneity by determining the mean deviation of these temperature differences divided by the standard error of these mean deviations ($k = \bar{d}/SE_{\bar{d}}$). At the two elevations being compared (1220 m and 1550 m), the heterogeneity was relatively great in 1971 ($k = 6.2$) and 1972 ($k = 7.5$), while in 1973 it is relatively homogeneous ($k = 0.8$). The differences in the patterns of low temperatures may be adequate to require an additional consideration of temperature in explaining the environmental correlations with the frequency of gene arrangement A. At present, however, we do not have sufficient data to pursue this suggestion so the two data points in 1971 have been excluded from this analysis. Clearly, we need more study along these lines for a better understanding of this problem.

ACKNOWLEDGEMENTS

Sincere thanks are extended to Dr. K. W. Bridges of Department of Botany for helpful criticism of the manuscript.

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